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DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN CERTAIN ROOTS¹

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INTRODUCTION

The subject of periodicity of growth activities in plants is by no means a new one; in fact, it is one of the oldest. But a careful review of the available literature shows that there are still certain phases of the work which have not yet been thoroughly investigated. Two of these are embodied in the present paper, *viz.*, rhythms of elongation and rhythms of cell division in roots under constant environmental conditions.

HISTORICAL

Elongation

Aerial parts. Sachs (29,30) gives an historical account of the older literature up to his time. No attempt will be made to reproduce it here except to point out that the work before his time was all done on large plants which grew rapidly and which in most cases had to be observed in the open where external factors could not be controlled. Hence, the work was only of the grossest nature and led to no definite general conclusions. In 1872 Sachs (29) published the results of his study of the elongation of the stem in various plants, including *Dahlia variabilis*, *Fritillaria*, *Polemonium*, etc. In general he found that plants exposed to the alternation of darkness and light exhibited a single daily wave of elongation in which the maximum occurred shortly after sunrise, and the minimum shortly after sunset. This he formulated into his so-called "universal law." He further found that this daily periodicity is entirely absent from plants grown continually in the dark.

In 1873 Prantl (27) found, in studying the rate of growth in leaves, that curves for increase in width are very similar to those for increase in length, and that under normal conditions the maximum is reached in the morning from 6 to 9 and the minimum in the evening from 6 to 9. He found, further, that by changing the hours of illumination and darkness he could shift the times of maxima and minima at will, since for each change in the time of illumination and darkness there was a corresponding change in the times of maxima and minima. These results show clearly that the daily periodicity here is an induced one. In continuous darkness this periodicity was absent. In 1878 Stebler (33) published the results of similar observations on the

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growth of leaves of various species among which were *Secale cereale*, *Triticum vulgare*, *Allium Ceba*, *Cucurbita*, *Melampsora*, etc. His results seem to show that the time of maximum growth coincides with the time of maximum light intensity and that of the minimum growth with the time of minimum light intensity. Here, also, a single daily wave of elongation and increase in width was found, though its precise relation to the time of environmental changes was somewhat different.

In 1879 Baranetzky (3) published the results of his investigations on a number of species including *Gesneria tubiflora*, *G. cardinalis*, *Helianthus tuberosus* (plants from tubers), *H. annuus*, *Brassica rapa*, etc. In brief, he found that plants which exhibit a regular daily periodicity when exposed to the alternation of darkness and light gradually lose this periodicity when placed in continuous darkness. The time required for complete loss varies from two to three days in the case of *Gesneria tubiflora* to 14 days in that of *Helianthus tuberosus*. Further, the intensity of the rhythms decreases from day to day. Plants grown from the beginning in darkness exhibited no periodicity except in the case of the shoots of *Brassica rapa*, some of which showed a very clear and regular rhythm, others a poorly defined one, and still others showed none at all. He regards this as due to heredity. It could hardly be considered such according to the commonly accepted use of the word heredity. A better term would be the "persistence of the habit" in the tuber, and its subsequent transference to the shoot.

In 1892 Godlewski (10) published the results of his researches on the growth of epicotyls of *Phaseolus multiflorus*. In the experiments carried out in June 1888, he found that plants growing under normal conditions exhibited a single daily wave of elongation, the maximum coming in the afternoon and the minimum near midnight. The following year plants grown from seeds of the same collecting showed the waves to come somewhat later, the maximum at evening and the minimum in the morning. Further experiments with seeds of a different lot gave two daily waves. Plants exposed to uniform conditions showed a very considerable variation. In some no marked rhythms were found, and in others rather irregular and unsteady ones were found.

Underground parts. The earliest work on underground parts was that of Strehl (36) in 1874, on the radicle of *Lupinus albus* L. The conditions of his experiments were, however, far from normal, inasmuch as the seedlings were grown with their roots in water and kept near a west window where they were exposed to moderately strong light. In plants thus subjected to the alternation of day and night he found in most cases a single daily wave of elongation with maximum coming near midnight and minimum near noon. In a few cases two waves were found.

In 1891 MacMillan (23) reported the results of his experiments upon the potato tuber. He found that tubers growing in continuous darkness exhibited rhythmic pulsations in their growth, showing two, three, and four

maxima and minima in the 24-hour period. He further found that the rhythms of the tuber were related to the periodicity of the aerial parts, but he thought it also probable that the tuber exhibited a rhythm of its own which was more or less obscured by the induced periodicity of the aerial parts. In 1901 Miss Gardner (9) reported the results of experiments on the growth of roots of *Pisum sativum* and *Vicia faba*. She found that roots exposed to the alternation of day and night elongated much more rapidly during the day than during the night. But the conditions of the experiment were far from normal, *viz.*, seedlings were placed in moist sawdust in wooden boxes with one glass face, and were made to grow in a horizontal direction.

A more recent work on elongation of underground parts is that of Kellcott (14) in 1904. In general, he found that curves for elongation of roots grown from bulbs of *Allium Cepa* exhibited three waves of elongation in the 24-hour period. Curves for different individuals were quite similar, though differing somewhat in the precise time of their maxima and minima. In general, the maxima came in the early morning and late afternoon and the minima came near noon and midnight. This work was done in the absence of any changes of environment, and hence is the first work definitely noting a regular rhythm not induced by external changes. A brief summary of the above account of investigations on elongation should note that (1) regular daily periodicity exists in the presence of regular daily changes in the environment; (2) this periodicity is gradually lost when the plants are exposed to constant conditions, though irregular and unsteady variations of the type called "autonomic" are reported; (3) the work of Kellicott on the root of *Allium* is the first to note any regularity in elongation of roots grown under constant conditions.

Cell Division

Lower plants. A great many statements are to be found in the older literature in regard to the time of day of nuclear and cell division among the Thallophytes. Thus Braun (4) notes that cell division in the formation of the gonidia of *Draparnaldia mutabilis* occurs between 6 A.M. and 11 A.M.; of *Stigeoclonium protensum*, between 6 A.M. and 10 A.M.; of *Cladophora tuberculata*, 8 A.M. to 2 P.M.; cell division in *Spirogyra* is most rapid during the night. Thuret (37) notes that the zoospores of *Vaucheria* are always liberated at about 8 A.M.; those of *Cutleria multifida* at daybreak; while those of *Enteromorpha clathrata* escape during the afternoon. Famintzin (8) corroborates Braun's statement in regard to *Spirogyra*. Strasburger (35) notes that cell division in *Spirogyra* is most rapid at 10-12 P.M., but may be delayed until the following morning if the plants are placed at a temperature of 0° to 5° C. during the night. De Wildeman (39), on the other hand, was unable to note any sensible difference, between day and night, in the rate of division in the cells of *Spirogyra*. His work was done

during the winter months from material collected outside. Kurssanow (17) reports *Zygnema* as dividing most frequently between 9 P.M. and midnight. Numerous other examples from the older literature are cited by Karsten (12) which will not be reproduced here. Karsten (13) in the most recent paper has shown that the desmids: *Cosmarium Botrytis*, *Closterium moniliferum*, and *Mesotaenium Endlicherianum*, when grown under normal conditions of illumination, exhibit a regular daily periodicity in the rate of nuclear and cell division. *Cosmarium* exhibits three waves. The primary maximum (about 50 percent of all cells) occurs at 1 A.M., with secondary maxima at 5 and 11 A.M. The primary minimum (about 5 percent of all cells) occurs at 1-3 P.M., with secondary minima at 3 and 7 A.M. Similarly, *Closterium* and *Mesotaenium* exhibit regular waves in the percentage of cells undergoing division, differing only in detail from the condition above noted for *Cosmarium*. It should be borne in mind that all of the above cited cases are reported from experiments carried on under normal conditions of light and darkness.

Aerial parts of higher plants. The only published reports on periodicity of cell division in aerial parts known to the writer are those of Karsten (12 and 13). He used as material the apical meristem of seedlings of *Pisum sativum*, *Zea Mays*, and *Pinus austriaca*. Seedlings of *Pisum* grown in continuous darkness showed a very marked increase in the number of cells undergoing division between 9:30 P.M. and 2 A.M., with a minimum falling at 6 A.M., while the remainder of the day was occupied with smaller fluctuations. Similarly, the curve for *Zea Mays* grown in continuous darkness shows numerous minor oscillations during the day, with a very marked rising during the night until the crest is reached at about 4 A.M., from which time it falls back again to the day position. *This rhythm is independent of changes in illumination and temperature.* The effect of alternation of darkness and light was then studied. When plants were lighted during the day and darkened during the night, much the same sort of curve was obtained as when in continuous darkness. When the times of illumination and darkness were reversed, two waves appeared with maxima at 6 A.M. and 6 P.M. and minima at 4 P.M. and 10 P.M. When the plants were continually lighted the waves were much shorter and more numerous. Seedlings of *Pinus austriaca*, when grown under normal conditions, showed maxima at 4 A.M. and 4 P.M. and minima at 12 M. and 6 P.M.

Underground parts of higher plants. The earliest work of this sort done on roots is that of Lewis (21). In the preliminary notice of this work it is shown that roots from bulbs of *Allium Cepa*, when grown in water and under normal conditions of illumination, *i.e.*, regularly alternating day and night, show two waves in their rate of cell division. The maxima come at midnight and noon, and the minima at 4 A.M. and 4 P.M. When yellow light was used the maxima appeared as before, but with the minima at 8 A.M. and 8 P.M. In blue light the maxima occurred at 4 A.M. and

noon, with the minima at 8 A.M. and 4 P.M. Finally, in continual darkness the maxima came at 4 P.M. and 8 A.M. with the minima at midnight and noon. Two waves were found in all these curves. The work of Kellicott (14) also shows two waves in the curves for cell division in roots of *Allium Cepa* grown from bulbs and in moist sawdust. The maxima came at 11 P.M. and 1 P.M. and the minima at 3 P.M. and 7 A.M. It should be noted that in his curve I no figures are given for 5 A.M., and that in his curve II the curve rises from the "normal" 11 P.M. maximum to a much higher one at 5 A.M. This point will be referred to again in connection with my own results. It should also be noted that a total difference of 13° C. appears between the highest and lowest temperatures, though there is apparently no direct relation to be noted in the curves between these temperature changes and changes in rate of cell division. Roots of *Podophyllum peltatum* also showed rhythms in their curve of cell division, though they were more numerous than in *Allium*.

Karsten (12) studied cell-division in the root tips of *Vicia faba* and *Zea Mays*. The curve for *Vicia faba* showed marked maxima at 9 A.M. and 9 P.M. with minima at 4 P.M. and 7 A.M., and a few minor variations. The curve for *Zea Mays* showed smaller oscillations throughout the entire 24-hour period, though the curve is higher from 5 A.M. to 6 P.M. and lower from 6 P.M. to 5 A.M., the highest point being reached at 7 A.M. and the lowest at 9 P.M. These experiments were conducted in continuous darkness.

Miscellaneous

It is of interest and indirect bearing on the present paper to mention a few other cases in which either rhythm or a daily periodicity is found. Pfeffer (26) found nearly the same results in regard to sleep movements of leaves, *viz.*, plants subjected either to constant illumination or to constant darkness lose their regular daily periodicity. In some cases autonomic waves are found under uniform conditions, and in others they are entirely absent. When present they show considerable variation both in different individuals and in different leaves of the same plant. Baranetzky (2) and Detmer (7) have shown that there is a single wave in the daily curve for root pressure. The maximum, while varying somewhat in different individuals, comes some time in the afternoon and the minimum about 12 hours later. In a recent paper, Romell (28) reports the same results from plants continually lighted: "Die Dauerlichtpflanzen, ohne Ausnahme, eine sehr ausgeprägte Tagesperiodicität in der Blütungskurve besäßen." Humphreys (11) calls attention to the presence of two maxima and two minima in daily atmospheric pressure, and in electrical potential. Similarly, Dechevrens (6) reports, from observations in Jersey, the presence of a diurnal rhythm in electrical potential of the atmosphere. Kraus (15, 16) and Millardet (24) have shown that the daily periodicity of tissue tension is gradually lost when the plants are exposed to uniform conditions. Finally,

Curtiss (5) has noted, under constant illumination, rhythms in the rate of transpiration of certain plants. A pronounced maximum occurs near midday, with other minor oscillations. He has further noted that the stomata are more responsive to stimuli in the morning than in the afternoon.

From the foregoing account of earlier work it is seen that in all cases when plants are exposed to the normal alternation of darkness and light a regular daily "periodicity" is thus induced; and that when these conditions are rendered uniform this "periodicity" is gradually lost. From the work of Kellicott (14), Karsten (12), and from the results of the present paper, it is seen that there is present, under uniform conditions, a "rhythm" which is entirely independent of the "periodicity" induced by environmental changes. This rhythm is concealed by the more prominent periodicity under normal conditions. Previous workers, including both Kellicott and Karsten, have failed to point out this difference. It is the object of this work to determine to what extent these rhythms are present in other species than those mentioned above, their probable cause, and their relation to the time of day.

MATERIALS AND METHODS

Materials

For the present study the following materials were used. radicles from seedlings of *Cucurbita Pepo* L., *Lupinus albus* L., *Pisum sativum* L., *Vicia faba* L., *Allium Cepa* L., and *Zea everta* Sturt.; and roots from germinating bulbs of *Allium Cepa* L., *A. canadense* L., and *A. cernuum* Roth.

Methods

Elongation. Seeds or bulbs were germinated in moist sawdust loosely packed in glass germinating chambers. These chambers had one face ground plane and polished, and measured 75 x 100 x 400 mm. The plane face was ruled in horizontal lines 2 mm. apart. Germination, except in a few cases, was secured at temperatures constant to within one degree C., though the temperatures used in different series ranged from 22° to 26° C. The cultural chambers were kept tilted a few degrees from the vertical while in the incubators, in order to have the root tips always growing directly along the inside of the chamber face. When observations were to be made, the chambers were taken from the incubators and placed before a horizontal microscope fitted with an eye-piece micrometer. The exact position of the tip of the growing root was then determined by measuring the number of micrometer spaces between it and the horizontal lines (on the face of the chamber) below and above it. In this way the exact position of the tip of the root was determined every hour throughout the course of the experiment, and the increments of growth calculated from the changes in this position. Since one eye-piece (micrometer) division was equal to 0.04 mm. absolute measurement, the growth increments could be measured accurately to 0.01 mm.

Cell Division. Root tips of the species to be studied were cut from seedlings (or germinating bulbs) germinated at 22° to 26° C. (but always constant to within one degree for any particular series) in moist sawdust in ordinary 4-inch pots. These tips were cut at intervals of two hours, 72 to 96 hours after the seeds or bulbs had been placed in the germinating pots. The tips were fixed 24 to 36 hours in medium chrom-acetic solution, washed, dehydrated, imbedded in paraffin in the usual way, cut into sections 10 microns in thickness, and stained in Delafield's haematoxylin. Only those slides showing sections cut exactly parallel to the long axis of the tip were used. Two or three slides were chosen for each hour, and from each slide chosen the median section and one on either side were marked off. The slides, having been previously labeled with a writing diamond, were now given a new number without regard to the first one, and all counting of dividing cells was done by this last number. The two numbers were not compared until the entire series had been counted, so that any influence due to a knowledge of the time of day of the particular slide being counted was avoided.

A typical observation. The slides and particular sections having been chosen for observation, the diameter of the section was then measured at a point where the root had attained a uniform diameter. Measurements were made by the eye-piece micrometer scale and are given accurate to the nearest 0.0085 mm. The diameter measured, the slide was moved by the mechanical stage to a point at a distance from the growing point of the tip equal to twice the diameter (measured where the root had attained uniform diameter) of the tip. The number of dividing cells in this area between the growing point and the imaginary line drawn across the section was then carefully counted. In order to facilitate the counting, a small rectangle was made by gluing four straight bristles (one for each side of the rectangle) into the eye-piece of the microscope. The section was then moved back and forth through this rectangle for counting. The number of cells dividing were recorded under the four phases: prophase, metaphase, anaphase, and telophase. All cells with nuclei between an evident spirem and the completion of the cell plate in the telophase were considered to be dividing. The area of the field observed was then determined by carefully counting the number of squares of a net eye-piece micrometer necessary to cover the field. This area was reduced to absolute measurement in square millimeters. It was soon discovered that the value so obtained very nearly approximated the value $7d^2/4$, where d equals the diameter of the section in millimeters. The amount of difference between the two methods mentioned above was always very small and constant for a given species. All calculations of areas given below were made from the latter formula.

Since it has been shown by a number of investigators, among whom are Amelung (1), Sanio (31), and, more recently, La Rue and Bartlett (18), that in corresponding organs of plants of the same species variation in cell

size is so slight that variations in size of the part are due almost entirely to differences in cell number, and not in cell size, the number of dividing cells in all cases was reduced to the proper proportion for a common constant area of one square millimeter. This thus avoided error due to observation of roots of different sizes. This care was taken by Kellicott (14), but was omitted by Karsten (12 and 13). In all cases the area observed contained practically all of the dividing cells.

INVESTIGATION

Elongation

Pisum sativum. Seeds of two varieties, viz., wrinkled (*gradus*), and smooth (No. 1 White Field of D. M. Ferry & Co.), were allowed to germinate, and when the radicles had attained a length of 50–70 mm. observations began. All observations were made in a dark room and at constant temperatures, so that the results obtained could not have been influenced by environmental changes of temperature and illumination. (In all the following plant and curve numbers it has been thought best to reproduce here the numbers as they actually occur in the original data). Space will permit the reproduction of but few of the mass of figures and curves upon which these results are based. Table 1 shows a representative set of elongation measurements; while in table 2 the times of maxima and minima of ten plants out of a total of 50 of this species studied are grouped. The other 40 are duplicates of one or other of those given in this table.

A study of curves 193 and 194 (figures in table 1) shows that elongation is rhythmic or oscillatory in nature, three waves of elongation occurring in the 24-hour period. Elongation is least rapid at 1–3 P.M., rises to a maximum at 5–7 P.M., with other maxima at 11 P.M. to 1 A.M., and 5–7 A.M., and minima at 9 P.M. and 3–5 A.M. These plants were of the smooth-seeded variety. Curve 174 again shows three waves of elongation; here, however, the maxima occur at 11 A.M., 9 P.M., and 5 A.M., and the minima at 7 P.M., 1 A.M., and 7 A.M. Curve 160 also shows three waves, with maxima at 1 P.M., 11 P.M., and 5 A.M., and minima at 11 A.M., 9 P.M., and 3 A.M. Comparison of these curves seems to show little uniformity. They are, however, not comparable for two reasons: (1) the first two are obtained from plants of the smooth-seeded variety and the latter two are from those of the wrinkled-seeded variety; (2) germination² in the case of the first two was begun at 9 A.M., in no. 174 it was begun at 8 P.M., and in no. 160 at 6 P.M. In order to make no. 174 comparable, with respect to time after initiation of activity, to plants started at 9 A.M., it will be necessary to move the entire curve (no. 174) backward 11 hours or forward 13 hours; similarly, no. 160 will have to be moved backward 9

² In all cases throughout this paper the time stated for beginning of germination is the time when seeds were placed in the germinating chambers.

TABLE 1. *Pisum sativum*. Elongation of Plants 193 and 194 (Smooth-seeded variety)

Time	Temp.	193		194	
10 A.M.	20.3	0.912		1.135	
11	20.8	0.955	1.867	1.045	2.180
12 M.	20.8	0.855		0.900	
1 P.M.	20.6	0.706	1.561	0.855	1.755
2	21.0	0.784		0.855	
3	21.0	1.116	1.900	0.765	1.620
4	21.0	1.180		1.045	
5	21.0	0.837	2.017	0.977	2.022
6	21.0	0.720		1.180	
7	21.2	0.675	1.395	1.135	2.315
8	21.0	0.315		0.900	
9	21.0	0.225	0.540	1.000	1.900
10	21.2	0.651		1.085	
11	21.0	0.457	1.108	0.865	1.953
12 N.	21.0	0.708		0.955	
1 A.M.	21.0	0.425	1.133	0.888	1.843
2	—	0.475		0.850	
3	21.0	0.475	0.950	0.850	1.700
4	21.0	0.750		0.600	
5	21.0	0.884	1.634	0.791	1.391
6	—	0.675		0.972	
7	21.0	0.675	1.350	0.972	1.944
8	—	0.585		0.522	
9	21.0	0.585	1.170	0.522	1.044

hours or forward 15 hours. The justification for this will be discussed later in connection with curves for cell division. The times of maxima and minima for plants given in table 2 are rewritten there on the basis of having started at 9 A.M. The lack of uniformity at first apparent disappears when

TABLE 2. *Pisum sativum*. Grouping of Maxima and Minima of Elongation, Wrinkled-seeded Variety

Plant	Maxima			Minima		
155. . .	12 M.	10 P.M.	4 A.M.	10 A.M.	8 P.M.	2 A.M.
159. . .	2 P.M.	10 P.M.	6 A.M.	10 A.M.	6 P.M.	4 A.M.
160. . .	2 P.M.	8 P.M.	4 A.M.	12 M.	6 P.M.	2 A.M.
165. . .	2 P.M.	8 P.M.	4 A.M.	10 A.M.	6 P.M.	2 A.M.
169. . .	4 P.M.	8 P.M.	6 A.M.	12 M.	6 P.M.	4 A.M.
170. . .	2 P.M.	10 P.M.	4 A.M.	12 M.	8 P.M.	6 A.M.
174. . .	6 P.M.	12 N.	10 A.M.	2 P.M.	8 P.M.	8 A.M.
175. . .	10 P.M.	12 N.	10 A.M.		4 P.M.	2 A.M.
	2 P.M.	12 N.	6 A.M.	8 A.M.	8 P.M.	12 N.

SUMMARY

Maxima 2-6 P.M. 8-12 P.M. 4-6 (10)³ A.M.
Minima 10 A.M.-2 P.M. 6-8 P.M. 2-4 (6)³ A.M.

Smooth-seeded Variety

Plant	Maxima			Minima		
193. . .	5 P.M.	1 A.M.	5 A.M.	1 P.M.	9 P.M.	3 A.M.
194. . .	7 P.M.	11 P.M.	7 A.M.	3 P.M.	9 P.M.	5 A.M.

³ Parentheses indicate an occasional variation in time to that enclosed by them.

the curves are plotted on an equal basis with respect to time after initiation of activity. Thus in general, in table 2, maxima occur at 2-6 P.M., 8-12 P.M., and 4-6 (10) A.M. in the wrinkled-seeded variety; and at 5-7 P.M., 11 P.M.-1 A.M., and 5-7 A.M. in the smooth-seeded variety; while the minima occur at 10 A.M.-2 P.M., 6-8 P.M., and 2-4 (6) A.M.; and 1-3 P.M., 9 P.M., and 3-5 A.M. respectively. It will be seen that the general character of the curves is the same for both wrinkled-seeded and smooth-seeded varieties. Both exhibit three waves of elongation in the 24-hour period, though the precise time of maxima and minima is usually slightly later in the smooth-seeded than in the wrinkled-seeded variety.

Except in a few cases, observations ceased at the close of the 24-hour period. In those few cases in which observations continued longer there was no material difference between the two days. The curve continued in the same oscillatory or rhythmic manner. The outstanding feature of these results is the rhythmic nature of elongation.

Lupinus albus. Seeds were germinated, and seedlings studied, in the

TABLE 3. *Lupinus albus*. Grouping of Maxima and Minima of Elongation

Plant	Maxima			Minima		
68...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	5 A.M.
69...	1 P.M.	1 A.M.	7 A.M.	11 A.M.	7 P.M.	5 A.M.
70...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	9 P.M.	3 A.M.
71...	3 P.M.	7 P.M.	5 A.M.	1 P.M.	5 P.M.	3 A.M.
72...	1 P.M.	9 P.M.	7 A.M.	1 P.M.	7 P.M.	5 A.M.
73...	3 P.M.	11 P.M.	7 A.M.	1 P.M.	5 P.M.	3 A.M.

SUMMARY

Maxima.....1-3 P.M. 7 P.M.-1 A.M. 5-7 A.M.

Minima.....11 A.M.-1 P.M. 5-9 P.M. 3-5 A.M.

same manner as above described for *Pisum*. In table 3 the maxima and minima of eight representative curves are grouped. A total of 23 different individuals was studied. It will be seen that here again three waves of elongation occur in the 24-hour period, with maxima at 1-3 P.M., 7 P.M.—1 A.M., and 5-7 A.M.; and minima at 11 A.M.—1 P.M., 5-9 P.M., and 3-5 A.M. Germination was begun at 9 A.M.

Curves 70 and 73 illustrate the character of elongation in two of these plants. While the corresponding waves (in regard to time of occurrence) in the various plants are not all of the same amplitude, the times of their maxima and minima are very close, and the character of the curves is very similar, indicating that once these activities are initiated they proceed in rhythmic fashion; and the time interval of the waves is a more or less nearly constant feature. The only earlier work on the root of *Lupinus* is that of Strehl (36). His results are not comparable with those of the present paper since his seedlings were exposed to the alternation of day and night, and hence any oscillations not induced by this alternation would be likely to be entirely concealed by the more prominent daily periodicity.

Allium Cepa. Roots from both germinating seeds and bulbs were used. The bulbs were uniform and of a medium-sized white variety, and the seed of the Yellow Danvers (D. M. Ferry & Co.) variety.

Roots from Bulbs. In table 4 are grouped the times of maxima and minima of the elongation of the roots of seven different plants. These are chosen to represent the various types of curves, and consequently show somewhat less approach to uniformity than when all curves are considered. Curves 272 and 296 show three waves of elongation in the 24-hour period. The maxima come at 7-9 A.M., 7 P.M., and 1 A.M.; and the minima at 1-3 P.M., 11 P.M., and 5 A.M. This type of curve is exhibited by about

TABLE 4. *Allium Cepa* (bulb). *Grouping of Maxima and Minima of Elongation*

Plant	Maxima			Minima		
263....	11 A.M.		1 A.M.	9 P.M.		3 A.M.
261....	7 A.M.	1 P.M.	9 P.M.	1 A.M.	9 A.M.	5 P.M.
264....	11 A.M.	5 P.M.	9 P.M.	5 A.M.	3 P.M.	7 P.M.
254....	9 A.M.	9 P.M.	1 A.M.	3 P.M.	11 P.M.	7 A.M.
271....	11 A.M.	7 P.M.	3 A.M.	5 P.M.	11 P.M.	5 A.M.
272....	9 A.M.	7 P.M.	1 A.M.	3 P.M.	11 P.M.	5 A.M.
296....	7 A.M.	7 P.M.	1 A.M.	1 P.M.	11 P.M.	5 A.M.

SUMMARY

Maxima.....7-11 A.M. 7-9 P.M. 1-3 (5) A.M.
 Minima.....1-5 P.M. 9-11 P.M. 3-7 A.M.

75 percent of the plants. Comparison with Kellicott's (14) curves shows only slight differences in the exact time of occurrence of maxima and minima. A second type of behavior is illustrated in curves 261 and 264 where four waves are found in the 24-hour period. Three of these waves correspond closely, in regard to time, to those of the other plants which show three waves. A third type of curve is that shown by plant 263 where but two waves are found in the 24-hour period. Kellicott (14, page 545, fig. 7, curve II) shows a similar curve with but two waves. Two plants out of a total of 50 showed this type of curve.

Roots from Seeds. Curves for elongation of roots from seedlings differ from those from bulbs mainly in that they are about equally divided between three- and four-wave types. In curves 275 and 288 three waves are shown, while curves 273, 274, and 276 exhibit four waves. All of these observations were made under identical conditions. Plants 275 and 276 grew beside each other in the same culture chamber, and a study of their curves shows how similar a four-wave curve is to one of three waves. It will be seen that the noon maximum comes two hours earlier in 276 than in 275, while the afternoon minimum comes two hours later in 276 than in 275. The other maxima, common to both, coincide; the difference in number of waves being due to the fact that 276 reaches its third maximum much earlier, sinks to a minimum, and then rises to a fourth maximum by the time 275

TABLE 5. *Allium Cepa* (Seed). *Grouping of Maxima and Minima of Elongation. Four-Wave Type*

Plant	Maxima				Minima			
276..	5 A.M.	11 A.M.	7 P.M.	1 A.M.	7 A.M.	5 P.M.	9 P.M.	3 A.M.
277..	9 A.M.	1 P.M.	7 P.M.	5 A.M.	11 A.M.	5 P.M.	1 P.M.	7 A.M.
273..	9 A.M.	1 P.M.	7 P.M.	1 A.M.	11 A.M.	5 P.M.	9 P.M.	3 A.M.
279..	9 A.M.	1 P.M.	7 P.M.	3 A.M.	11 A.M.	3 P.M.	9 P.M.	7 A.M.
283..	9 A.M.	1 P.M.	7 P.M.	3 A.	11 A.M.	3 P.M.	9 P.M.	7 A.M.

SUMMARY

Maxima.....5-9 A.M. (11 A.M.) 1-3 P.M. 7 P.M. 1-5 A.M.

Minima.....(7) 11 A.M. 3-5 P.M. 9 P.M. (1 A.M.) 3-7 A.M.

has attained its third maximum. A similar comparison of curves 281 and 283 shows again how similar in general character are the curves of the two types. In curve 276 the extra wave appears in the hours just preceding and just following midnight, while in 283 the extra wave is only a very low-crested one and appears during the forenoon. In table 5 the maxima and minima of five different curves of the four wave type are grouped. It is seen that these curves are very similar and that there is very little overlapping of times of maxima and minima. In table 6 the summary of these

TABLE 6. *Allium Cepa* (Seed). *Comparison of Maxima and Minima of Elongation in Four-wave Curves with those of Three Waves*

		Maxima			
Four-wave type					
See table 5		5-9 A.M.	11 A.M.-3 P.M.	7 P.M.	1-5 A.M.
Three-wave type					
275		5 A.M.	1 P.M.	7 P.M.	
280			11 A.M.	5 P.M.	3 A.M.
286		5 A.M.	11 A.M.	7 P.M.	
288		7 A.M.		5 P.M.	11 P.M.
281		5 A.M.	9 A.M.	5 P.M.	
		Minima			
Four-wave type					
See table 5		(7) 11 A.M.	3-5 P.M.	9 P.M. (1 A.M.)	3-7 A.M.
Three-wave type					
275			3 P.M.	1 A.M.	7 A.M.
280			1 P.M.	9 P.M.	7 A.M.
286			5 P.M.	9 P.M.	7 A.M.
288		9 A.M.		7 P.M.	5 A.M.
281			3 P.M.	11 P.M.	7 A.M.

curves is compared with five different curves of the three-wave type. It will thus be seen that the three-wave curves are, as individuals, very similar to those of the four-wave type, but differ among themselves primarily as to which of the waves (present in the four-wave curves) is omitted. The seeds for this work began germination at 9 A.M.

Cucurbita Pepo. Space will not permit so extensive a discussion as given above for *Pisum*, *Lupinus*, and *Allium*. Nothing unlike what we have already seen above was found in the study of this species. Curves

III and II2, out of a total of 14 different plants studied, are given on Plate XXIV. In these, also, three waves of elongation occur in the 24-hour period.

Zea everta. For this study the White Rice (D. M. Ferry & Co.) variety was used. A single curve is shown on Plate XXIV for elongation. Too little work was done on this species to warrant definite conclusions. The curve, 102, shows two waves of elongation in the 24-hour period.

Summary for Elongation. Summarizing briefly in regard to elongation, we find that (1) elongation in all plants studied proceeds in a wave-like fashion, two to four waves being exhibited in the 24-hour period; (2) there is more or less variation among the various individuals of the same species in regard to the precise time of day of the occurrence of maxima and minima, though these can be arranged into definite groups which show very little overlapping of time (see tables 2-6); (3) it is indicated, though not definitely proven, in the case of *Pisum*, that the precise time of the occurrence of maxima and minima depends upon the time when germination was begun, and shows no relation to the actual time of day. This latter point will be taken up and definitely proven in connection with rhythms in cell division. This fact, if true, might also account for a great deal of the variation in elongation curves of plants of the same species placed in the germinating chambers at the same time, since it is possible that some of the seeds may have coats that are more permeable to water than others, and hence the precise time of initiation of metabolic activity would vary slightly.

Cell Division

Pisum sativum. For this work root tips from both the wrinkled-seeded and smooth-seeded varieties of peas were used. Curve 2 (figures in table 7) shows results obtained from a study of the wrinkled variety. Seeds were placed in germinating pots at 9 A.M. at a temperature of 25° C. and allowed to germinate for 72 hours. The radicles had attained a length of 20-50 mm. when killing and fixing began. It will be seen that three waves of cell division occur in the period of 24 hours. The three maxima come at 1 P.M., 5 P.M., and 5 A.M.; and the minima come at 11 A.M., 3 P.M., and 9 P.M. The two maxima coming at 5 P.M. and 5 A.M. are about equal in extent. It will be noticed throughout the curves that follow that those waves in the various curves from roots of the same variety of seed which are coordinate in regard to time of appearance, are not always of the same amplitude. Kellicott (14) found similar results in *Podophyllum peltatum*. A study of the figures from which this curve is drawn (table 7) shows remarkable uniformity of the different roots for the same hour. Only at 5 and 11 A.M. do any appreciable differences occur, and then they are of such a nature that they do not affect the character of the curve. Curve 27 shows results from a similar study of the smooth-seeded variety. These seeds

TABLE 7. *Pisum sativum*. Wrinkled-seeded Variety. Figures for Cell Division, Curve 2. Germination began at 9 A.M. January 24-25, 1918

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C ⁶	Ave. 1 Tip	Ave. 3 Tips				
			Pro.	Meta.	Ana.	Telo.								
9 A.M...	25.0	.748 ⁴	160	47	11	34	252	257	284	259				
		.977 ⁶	180	49	8	36	273	279						
			233	47	7	23	310	317						
			.935	262	63	8	35	368	240		241			
		1.529	239	52	13	44	348	227						
			263	66	10	55	394	257						
			.748	190	45	8	25	268	274		252			
		.977	147	40	9	32	228	233						
			166	43	5	32	246	251						
		11 A.M...	25.0	1.03	107	42	3	9	161		87	98	139	
				1.856	108	37	4	14	163		88			
					168	38	4	15	225		121			
	.858			112	50	12	46	220	168	138				
1.188	83			25	7	35	150	115						
	98			40	10	28	176	135						
	.901			188	61	6	33	288	203	180				
1.421	132			55	6	30	223	157						
1 P.M...	26.0			.935	249	50	16	37	352	230	278	341		
				1.529	351	61	12	32	456	301				
					320	51	9	36	416	272				
			.867	327	39	5	29	400	304	319				
		1.315	320	43	11	38	412	313						
			370	43	6	25	444	337						
			8.42	306	62	13	66	447	361	339				
		1.237	288	58	13	55	414	334						
			272	75	8	45	400	323						
		3 P.M...	26.0	.875	221	34	4	29	288	214	240		267	
				1.340	288	36	12	22	358	265				
					239	42	17	27	325	242				
	.859			292	51	14	28	385	295	294				
1.290	307			54	9	28	398	305						
	285			44	10	32	371	284						
5 P.M...	25.5			.988	516	87	14	62	679	396	405	397		
				1.707	598	62	11	50	721	421				
					519	80	31	66	696	406				
					.918	507	46	10	56	619	419			367
				1.475	412	59	9	41	521	352				
					374	59	16	38	487	330				
			.825	368	63	10	46	487	408	419				
		1.189	368	67	18	33	486	408						
			414	54	12	44	524	440						

⁴ Diameter of section in millimeters, always upper number.⁵ Area counted, see page 386.⁶ C = Constant necessary for reduction of figures to common area of 1 sq. mm.

TABLE 7 (Continued)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C	Ave. 1 Tip	Ave. 3 Tips
			Pro.	Meta.	Ana.	Telo.				
7 P.M. . .	25.25	.867	375	68	15	64	522	397	403	368
		1.315	367	83	11	55	516	392		
			387	88	19	60	554	421		
		.782	325	49	8	49	431	402	375	
		1.070	328	41	9	33	411	384		
			278	43	8	33	362	338		
		.850	282	64	14	51	411	326	327	
		1.261	324	62	11	59	456	361		
			242	62	15	53	372	295		
9 P.M. . .	25.25	.833	156	52	11	34	253	208	226	237
		1.212	182	44	7	40	273	225		
			191	55	12	40	298	246		
		.910	204	49	11	43	307	211	204	
		1.448	178	57	11	47	293	203		
			170	54	14	47	285	197		
		.842	237	65	10	36	348	281	280	
		1.237	256	66	10	30	362	292		
			227	67	6	32	332	268		
11 P.M. . .	25.0	.884	301	56	9	30	396	289	284	271
		1.368	309	43	21	35	408	298		
			277	49	13	25	364	266		
		.774	231	56	7	42	336	321	316	
		1.047	231	61	12	38	341	325		
			200	63	17	38	318	303		
		.833	148	42	7	22	219	180	213	
		1.212	196	33	12	37	278	229		
			189	37	8	45	279	230		
1 A.M. . .	25.0	.816	255	55	12	40	362	311	315	295
		1.165	326	64	11	38	349	299		
			273	65	10	45	393	337		
		.842	274	60	10	37	381	308	316	
		1.237	274	59	10	56	399	322		
			260	70	7	57	394	318		
		.910	246	59	8	48	361	249	256	
		1.448	257	85	6	23	371	256		
			271	58	18	34	381	263		
3 A.M. . .	25.0	.979	382	53	5	15	455	272	265	301
		1.677	322	37	12	24	395	237		
			376	57	8	30	472	276		
		.807	343	45	10	30	428	375	365	
		1.140	291	56	12	24	383	336		
			331	61	20	28	440	385		
		.884	355	40	4	31	653	314	272	
		1.369	258	38	9	20	023	237		
			270	45	10	39	44	266		

TABLE 7 (*Concluded*)

Time	Temp.	Diam. Area	Dividing Cells				Total	Total × C.	Ave. 1 Tip	Ave. 3 3 Tips			
			Pro.	Meta.	Ana.	Telo.							
5 A.M. . .	25.0	.988	445	53	15	36	549	319	279	381			
		1.707	319	52	12	47	430	251					
			345	66	19	30	460	268					
		.918	497	71	23	39	630	427	438				
		1.475	510	87	25	59	681	461					
			455	89	23	66	633	428					
		.850	456	56	16	44	572	452	425				
		1.261	360	64	12	37	473	375					
			437	74	14	45	570	450					
		7 A.M. . .	24.0	.808	275	50	11	52	388		340	341	305
				1.140	311	60	12	38	421		369		
					258	53	13	33	357		313		
.859	240			57	10	53	360	278	279				
1.290	253			63	11	52	379	292					
	238			65	2	40	345	266					
.791	218			46	14	36	314	285	294				
1.093	221			59	6	47	333	302					
	202			55	12	56	325	295					

were placed in germinating pots at 9 A.M. and incubated for 72 hours at a temperature of 22°–23° C. Here also it will be seen that three waves occur in the 24-hour period. The maxima come at 3 P.M., 9 P.M., and 1 A.M.; and the minima at 11 A.M., 7 P.M., and 11 P.M. A comparison of curves 2 and 27 shows that the first two maxima of curve 2 each come just eight hours earlier (or 16 hours later) than two of curve 27, while the third maximum departs somewhat from this time relation. A similar relation exists between the minima.

Let us now turn to evidence in support of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity and not to time of day. Curve 28 is the result obtained from root tips of the smooth-seeded variety grown at the same time and in the same incubator as those represented by curve 27, with the difference that the seeds for curve 28 were placed in the germinating pots at 2 P.M., instead of 9 A.M. of the same day. In curve 28 three marked maxima occur with a very small fourth. Omitting, for the present, this extra small wave, we find maxima occurring at 7 P.M., 3 A.M., and 7 A.M., and minima at 3 P.M., 11 P.M., and 5 A.M. Now it will be seen that these seeds were started to germinate just 5 hours later than those of curve 27. Since root tips were cut and fixed every two hours, a difference of precisely five hours would not appear in the curves as such, but rather as a four- or six-hour difference. Comparison of the two curves will show that the 7 P.M. maximum of curve 28 is just four hours later than the 3 P.M. maximum of curve 27; similarly, the 3 P.M. and 11 P.M. minima of curve 28 are just 4 hours later than the

11 A.M. and 7 P.M. minima of curve 27; while the 3 A.M. and 7 A.M. maxima, and the 5 A.M. minimum of curve 28 are each just 6 hours later than the corresponding maxima and minimum of curve 27. Thus the entire curve 27 is earlier than curve 28 by an amount of time equal to the difference in time between the beginnings of germination. As further evidence on this point, a third series of root tips were cut at the same time and under identical conditions. The seeds for this third series were placed in the germinating pots at 8 P.M. Curve 31 shows the results of this study. In curve 28 a fourth wave was merely indicated, while in curve 31 there are definitely and clearly four waves. It is seen that because of the difference between the times when seeds were placed in germinating pots there would be expected to be a difference of just eleven hours between the times of initiation of activity in curves 27 and 31, and six hours between curves 28 and 31. Table 8 shows the maxima and minima of these curves correlated in respect to time (after initiation of activity) of their occurrence.

TABLE 8. *Pisum sativum*. Correlation of Maxima and Minima of Curves 27, 28, and 31

27	28		31		
Germination Began at 9 A. M.	Germination at 2 P. M.	Diff. from 27; 5 Hrs.	Germination at 8 P. M.	Diff. from 27; 11 Hrs.	Diff. from 28; 6 Hrs.
Maxima					
3 P.M.	7 P.M.	4	3 A.M.	12	8
9 P.M.	3 A.M.	6	7 A.M.	10	4
1 A.M.	7 A.M.	6	3 P.M.	14	8
	11 A.M.				
Minima					
11 A.M.	3 P.M.	4	9 P.M.	10	6
7 P.M.	11 P.M.	4	5 A.M.	10	6
11 P.M.	5 A.M.	6	11 A.M.	12	6
	9 A.M.		1 A.M.		

A study of this table shows that the same relation exists between curves 28 and 31, and 27 and 31, as is shown above between curves 27 and 28, *viz.*, there are in both curves 28 and 31 waves corresponding, in time after initiation of activity, to each of the three waves shown in curve 27. The extra (fourth) waves appearing in curves 28 and 31 not only do not have a corresponding wave in curve 27, but also seem not to be correlative to each other.

A further experiment of this same nature was carried out in which two series of peas of the smooth-seeded variety were placed in germinating pots at 9 A.M. and incubated at 24–25° C. for 48 hours. They were then removed from the incubators to a refrigerator where a recording thermometer showed the temperature to vary between 6.0° and –0.5° C. for a period of 48 hours. During the time of refrigeration, control plants were kept growing in the glass culture chambers used for elongation studies, and their elongation was measured. The elongation figures (omitted for lack of space) show that the temperature was sufficiently low to inhibit all but the slightest

activity. After the plants had been in the refrigerator for nine hours, and from that time until the end of the period of refrigeration, the amount of elongation of the individual plants ranged from 0.018 to 0.079 mm. per hour. In six hours after being taken from the refrigerator and incubated at a temperature of 24°–25° C. these same control plants had regained their normal rate of elongation for that temperature. At the end of the refrigeration period the seedlings from which root tips were to be cut were also incubated at a temperature of 24°–25° C. Series 33 (curve 33) was removed from the refrigerator at 9 A.M., and series 35 (curve 35) was removed at 1 P.M. A comparison of the two curves (table 9) shows that there are present, again,

TABLE 9. *Pisum sativum*. Correlation of Maxima and Minima of Curves 33, 35, and 27

33	35		27		
Removed from Refrigerator at 9 A. M.	Removed from Refrigerator at 1 P. M.	Diff. from 33	Germination Began at 9 A. M.	Diff. from 33	Diff. from 35
Maxima					
5 P.M.	9 P.M.	4	3 P.M.	2	6
11 P.M.	1 A.M.	2	9 P.M.	2	4
5 A.M.	9 A.M.	4	1 A.M.	4	8
Minima					
1 P.M.	7 P.M.	6	11 A.M.	2	8
9 P.M.	11 P.M.	2	7 P.M.	2	4
1 A.M.	5 A.M.	4	11 P.M.	2	6

three waves, and that the times of two of the maxima and one of the minima are just four hours later in curve 35 than in curve 33, while the 7 P.M. minimum of curve 35 is six, instead of four, hours later than the 1 P.M. minimum of curve 33; and that the 11 P.M. minimum and 1 A.M. maximum of curve 35 are each but two hours later than the corresponding minimum and maximum of curve 33. Hence, in general, these curves also differ from each other by a time interval equal to the difference in time between their initiation of activity after refrigeration.

A comparison of curves 33 and 27 (table 9) shows that with but one exception the maxima and minima of curve 33 occur just two hours later than the corresponding waves of curve 27. This exception is found where the 5 A.M. maximum of curve 33 comes four, instead of two, hours later than the 1 A.M. maximum of curve 27. While the particular amount of difference in time between waves in curves 33 and 27 has no special significance, the fact that the time interval between waves of one curve is the same as that between waves of the other curve, taken together with the relation we have just seen existing between all these other curves of *Pisum*, proves that these rhythms are regular and definite and not mere chance variations. It further indicates the truth of the contention that the time of occurrence of maxima and minima is related to the time of initiation of activity, and not to actual time of day.

We note from this study of cell division in *Pisum* that (1) once activity is

initiated it proceeds in a rhythmic fashion; (2) in general, three waves are shown in the 24-hour period; (3) the exact time of appearance of maxima and minima is dependent upon the time of initiation of activity and shows no relation to time of day.

Lupinus albus. Curves 1 and 13 show the results of a study of cell division in this species. These curves, again, show three waves. Curve 1 shows the first maximum and minimum coming about four hours earlier than the corresponding wave in curve 13, though the general character of the two curves is strikingly similar and their rhythmic nature is well demonstrated. It should be mentioned that the two curves were obtained from seeds of different lots. The seeds in both cases began germination at 9 A.M.

Allium Cepa, Roots from Bulbs. Curve 10 shows three waves of cell division with maxima coming at 1 P.M., 9 P.M., and 5 A.M.; and the minima at 3 P.M., 1 A.M., and 7 A.M. In comparing this curve with those given by Kellicott (14) it is found that the 1 P.M. maximum and the 3 P.M. and 7 A.M. minima correspond to maximum and minima at similar times in his curves; while the 9 P.M. maximum of curve 10 comes just two hours earlier than the 11 P.M. maximum of his curve I, and one hour later than the 8 P.M. maximum of his curve II (page 563 of his paper). The 1 A.M. minimum and 5 A.M. maximum of curve 10 find no equivalents in his curve I. In his curve III, however, a third maximum occurs at 5 A.M. It should be noted that no figures are given for 5 A.M. in his curve I, and hence it is possible that a third maximum may have been missed at this hour. Curve 24 is drawn from data obtained a year after that of curve 10, and from a different lot of bulbs. Other conditions were the same in both. In comparison it is seen that the noon maximum of curve 24 comes at 11 A.M. instead of 1 P.M.; the afternoon minimum comes at the same time as in curve 10; while an additional low-crested wave, with maximum at 5 P.M. and minimum at 7 P.M., appears between the times of the first and second waves of curve 10. The remaining waves are the same in both. Curve 24 thus shows four waves instead of the usual three. In comparing these curves with those of Kellicott's on *Allium* we note that the main difference is the larger number of waves here shown. Kellicott used much lower temperatures than those used in the present work, and it is possible that this may account for the smaller number of waves found in his curves.

Roots from Seeds. Curve 12 shows results from a study of roots from seeds of the Yellow Globe variety. It will be seen that there is little difference between this and curve 10 (from bulbs), three waves being found in each case. The essential difference is found in the fact that the curve does not drop so suddenly to a minimum after both the 1 P.M. and the 9 P.M. maxima, in curve 12, as does curve 10.

Zea everta. Curve 7 shows results obtained from a study of roots from seedlings of the White Rice variety. Germination began at 9 A.M. It will be seen that the curve is much more oscillatory in character. Karsten

(12) found much the same condition in *Zea Mays*. While the number of waves found in the 24-hour period is higher than in the case of any other species studied, yet the fact that mitotic activity proceeds in waves or rhythms is none the less clearly demonstrated.

Vicia faba. Curve 5 shows results obtained from a study of roots of *Vicia faba*. Germination began at 9 A.M. It will be seen that two waves of cell division occur in the 24-hour period. Maxima occur at 5 P.M. and 7 A.M. and minima at 1 P.M. and 1 A.M. Comparison of this curve with the figures given by Karsten (12, page 9) shows that he, too, found two extensive waves of cell division with maxima coming at 10 A.M. and 9 P.M., and minima at 4 P.M. and 7 A.M. Thus the maxima of curve 5 come just three and four hours earlier, and the minima three and six hours earlier, than in Karsten's results. Besides the two more extensive waves it will be seen that his figures show two very small waves, one coming in each larger wave. He, however, did not take into consideration variations in size of the sections counted, and this, taken together with a possible difference in time of beginning germination, probably accounts for the differences between his results and those of the present paper.

Allium cernuum. For this study bulbs were collected in the field in October, stored in boxes of soil, and kept in the open until ready for use the following January. Upon germination each bulb produced from two to four roots. Curve 23 shows results from this study; it will be seen that four very marked waves occur in the 24-hour period.

Allium canadense. For this study the small aerial bulblets were collected in October and stored in a dry, cool place until ready to be used the following January. Curve 22 shows results from this study. It will be seen that five waves of cell division occur in the 24-hour period.

A brief summary of the results obtained from this study of cell division shows the following facts: (1) the curve of cell division in all plants studied exhibits a number of oscillations in the 24-hour period, in the majority of plants three; (2) the exact time of occurrence of maxima and minima is dependent upon the time of initiation of activity and not on time of day.

RELATION BETWEEN ELONGATION AND CELL DIVISION

Historical

De Wildeman (39) has shown by exact measurements that cells of *Spirogyra* do not elongate during mitosis, while in the staminal hairs of *Tradescantia* there is very slight elongation of the cell during early prophases but none at all during the later stages. Ward (38) has shown in his study of cell division and elongation of filaments of *Bacillus ramosus* Fraenkel that elongation proceeds in a wave-like fashion and that "the period of cell division entails more or less cessation of growth." Kellicott (14) has shown that, in general, the same thing is true of elongation and cell division

in roots from bulbs of *Allium Cepa*, *i.e.*, the times of maxima of cell division are near the times of minima of elongation and *vice versa*. It should be noted that the observations of de Wildeman (39) and Ward (38) were made directly upon the dividing cell while it *was* dividing. The two processes were observed in one and the same cell. Such direct observation in the case of root tips is, of course, out of the question.

Experimental

Pisum sativum. In table 10 the times of maxima and minima of elongation and cell division in *Pisum* are compared. It is seen that in both the wrinkled-seeded and smooth-seeded varieties the times of maxima of elon-

TABLE 10. *Comparison of Maxima and Minima of Elongation and Cell Division in Pisum*
WRINKLED VARIETY

Elongation Maxima (see table 2)	2-6 P.M.	8-12 P.M.	4-6 (10) A.M.
Cell Division Minima (see curve 2)	3 P.M.	9 P.M.	11 A.M.
Elongation Minima	10 A.M.-2 P.M.	6-8 P.M.	2-4 (6) A.M.
Cell Division Maxima	1 P.M.	5 P.M.	5 A.M.

SMOOTH VARIETY

Elongation Maxima (see table 2)	5-7 P.M.	11 P.M.-1 A.M.	5-7 A.M.
Cell Division Minima (see curve 27) . . .	11 P.M.	11 P.M.	7 A.M.
Elongation Minima	1-3 P.M.	9 P.M.	3-5 A.M.
Cell Division Maxima	3 P.M.	9 P.M.	1 A.M.

gation correspond very closely to the times of minima of cell division, and *vice versa*. A single exception is found in each variety: in the wrinkled-seeded variety the 11 A.M. minimum of cell division comes considerably later than the corresponding maximum of elongation in the majority of plants; and in the smooth-seeded variety the 11 A.M. minimum of cell division comes much earlier than the corresponding maximum of elongation. With the exception of this one divergence in each case there is a very close reciprocal relation existing between the rapidity of elongation and the number of cells undergoing division.

TABLE 11. *Comparison of Maxima and Minima of Elongation and Cell Division in Lupinus*

Elongation Maxima (see table 3)	1-3 P.M.	7 P.M.-1 A.M.	5-7 A.M.
Cell Division Minima			
Curve 1	3 P.M.	1 A.M.	5 A.M.
Curve 13	7 P.M.	1 A.M.	7 A.M.
Elongation Minima	11 A.M.-1 P.M.	5-9 P.M.	3-5 A.M.
Cell Division Maxima			
Curve 1	9 A.M.	11 P.M.	3 A.M.
Curve 13	1 P.M.	11 P.M.	3 A.M.

Lupinus albus. In table 11 the maxima and minima of elongation and cell division in *Lupinus* are compared. It will be seen that here again there

is a very close reciprocal relation existing between elongation and cell division. A single large divergence occurs in the case of the 7 P.M. minimum of cell division in curve 13.

Allium Cepa. In table 12 the maxima and minima of elongation and cell division in *Allium Cepa* are compared. In the case of roots from bulbs we find, again, very nearly a reciprocal relation between rapidity of elongation and number of cells undergoing division. Another divergence is seen in the case of the 3 P.M. minimum of cell division in both curves 10 and 24 (or 7-9 P.M. maximum of elongation).

TABLE 12. *Comparison of Maxima and Minima of Elongation and Cell-Division in Allium Cepa*

ROOTS FROM BULBS			
Elongation Maxima (see table 4).....	7-11 A.M.	7-9 P.M.	1-3 (5) A.M.
Cell Division Minima			
Curve 10.....	7 A.M.	3 P.M.	1 A.M.
Curve 24.....	7 A.M.	3 P.M.-7 P.M.	1 A.M.
Elongation Minima.....	1-5 P.M.	9-11 P.M.	3-7 A.M.
Cell Division Maxima			
Curve 10.....	1 P.M.	9 P.M.	5 A.M.
Curve 24.....	11 A.M.-5 P.M.	11 P.M.	5 A.M.
ROOTS FROM SEEDS			
Elongation Maxima (see table 5).....	5-9 A.M.-(11 A.M.)	1-3 P.M.	7 P.M. 1-5 A.M.
Cell Division Minima			
Curve 12.....	7 A.M.	5 P.M.	3 A.M.
Elongation Minima.....	11 A.M.	3-5 P.M.	9 P.M. 3-7 A.M.
Cell Division Maxima.....	1 P.M.		9 P.M. 5 A.M.

In the case of roots from seeds all of the maxima and minima of cell division find corresponding minima and maxima respectively in elongation so that the reciprocal relation here is quite evident except for the extra fourth wave in elongation.

In general we may say that the times of maxima of elongation are near the times of minima of cell division and *vice versa* in all plants studied. This reciprocal relation is not so clearly expressed as in the case where both processes may be observed at the same time and in the same individual cell as Ward (38) found in *Bacillus ramosus* Fraenkel and de Wildeman (39) found in *Spirogyra*; but is probably as near as might be expected from the fact that the two processes must be observed, not only in different cells, but also in different individual roots.

DISCUSSION

The question naturally arises: What are the causes of the rhythm found both in the elongation and the cell division of the plants studied? That it may be due to external influences of changes in illumination and temperature

is out of the question, since this work was done in a dark room and the temperature was kept constant, except in a few cases, to within one degree. It seems quite clear, also, that it is not due to heredity, in the case of seedlings, as Semon (32) and Karsten (12) believed, since it has been shown by a number of earlier investigators that plants placed in continuous darkness and uniform temperatures gradually lose the periodicity which they had exhibited when exposed to the alternation of darkness and light. Now it would be expected that these rhythms would show some relation to the normal changes of night and day, even though the experimental plants were not so exposed, if the rhythms were due to the hereditary persistence of such effects upon the parent plants. It has been shown, however, in the case of *Pisum sativum* seedlings, that these rhythms have no relation to time of day, but rather that they depend, for the precise time of their appearance, upon the time of day when metabolic activity is initiated. It was at first thought that the rhythm might be due, in the case of germinating bulbs, to the persistence of a habit acquired by the bulb, while the bulb was itself growing and so exposed to the alternation of darkness and light, and the subsequent transfer of this habit to the growing parts. This is also disproved, since roots grown from seeds, in the case of *Allium Cepa*, exhibited the same rhythms as those grown from bulbs. That the rhythms of elongation and cell division may have a relation to the diurnal rhythms in atmospheric pressure and electrical potential is also out of the question, since it has been shown that the time of the waves in elongation and cell division depends upon the time of the initiation of metabolic activity, and that they vary according to the time when germination is begun, regardless of atmospheric conditions. Stoppel (34) found a relation existing between curves for sleep movements of plants and electrical potential of the atmosphere.

The two processes, growth and cell division, must necessarily go hand in hand as two of the vital activities of germinating seedlings. Just what the precise relation between them is, is not so definitely known, though it is quite evident that a certain size of the cell must be attained before cell division ensues, since cells from corresponding parts of different individuals of the same species vary but little in size. In a comparison of the curves for elongation and cell division it is seen that a general reciprocal relation exists between these two processes whereby there is a slowing-up in the rate of elongation at the time when there is the largest number of cells undergoing mitosis. The fact that the processes of elongation and cell division show such a reciprocal relation to each other within the individual cell is not so difficult to understand, since there is probably not enough energy available to permit both processes to go on at their maximum at the same time. It is to be recalled, however, that the zone of most extensive elongation in the root is not the same as the zone of mitotic activity (practically all mitoses occur within a zone bounded by the growing point and an imaginary line

across the section back from the growing point a distance equal to twice the diameter of the root). This reciprocal relation between elongation and cell division in the root as a whole might be explained on the same basis as that in the individual cell, provided there is a coordination within the root tip sufficient so that when a large number of cells are undergoing mitosis the total energy available within the tip is directed more to mitosis than toward growth and elongation, and hence the one process will be near its maximum when the other is near its minimum. Whether it be a matter of available energy or not, the fact remains that the two processes, elongation and cell division, do alternate with each other, both in the individual cell and in the root as a whole. Since neither process can go on for any considerable length of time to the exclusion of the other, the curve representing the extent of either will show waves such as those found in the present work. Thus, activity once initiated by the beginning of germination of the seed or bulb, these two processes, of necessity having a definite relation to each other, bring about the rhythms here found.

The fact that these rhythms have a definite interval in the various series of the same variety of seedlings, and that corresponding waves in the different series bear the same relation to each other as the time interval between the times of initiation of metabolic activity, *i.e.*, that the maxima and minima in the different curves depend for the time of their appearance upon the time when germination was begun, indicates that the ultimate cause of this alternation between mitosis and elongation is entirely an internal cause and not related to external conditions and is in perfect accord with the above suggested energy hypothesis. This harmony in the various series of plants of the same variety shows, further, that the rhythms here found are not mere chance variations in activity which, when plotted, show such curves, but rather that the two processes, elongation and cell division, follow each other in a regular manner, the root tip being occupied with one and then with the other, and hence showing a regular and definite oscillation from the one to the other.

Whether or not this reciprocal relation existing between elongation and cell division is sufficient entirely to account for these rhythms, and whether there might not also be other rhythms independent of, and more or less confused with, these first rhythms, is a question not satisfactorily answered by the data at hand. The fact that the times of maxima of elongation in a few cases did not coincide with the times of the minima of cell division might seem to indicate that there were other factors influencing the course of these activities in the plant besides the alternation of elongation and cell division. It is conceivable that a relation might exist between growth activity (including mitosis) and available food supply, whereby these metabolic processes might, once initiated, gradually increase and finally outweigh the capacity of the enzymes to render stored food available. Then, with a lessening proportion of available food, a slowing down of these processes

must ensue until the food supply is again adequate, after which the same processes may be repeated. In other words, may there not be a certain inertia inherent in these vital processes, so that once they are in operation a certain force is required to check them, and, once slowed down, a certain force is again required to accelerate them? This might explain oscillations in either process independently of the other, or in the sum of the two processes, but it would not explain the reciprocal relation between the two processes. The possibility of growth rate exceeding that of enzymatic activity is apparent in the exhaustion effects found at higher temperatures in seedlings of *Zea Mays* and *Pisum sativum* by Lehenbauer (19) and Leitsch (20).

It is necessary, also, to distinguish between the terms "periodicity" and "rhythm." By "periodicity" the earlier workers meant a regular oscillation which was caused by the alternation of day and night or by other external changes, and which was lost when the environmental conditions were rendered constant; while the term "rhythm" in the present paper is restricted to mean any oscillation in activity which is definite and regular and not related to any external influences. Thus these roots in their development exhibit "rhythms" in the absence of changes in environment, but not a "periodicity" in the sense in which the older writers used the term.

SUMMARY

1. Under constant uniform conditions elongation in all plants studied proceeds in a rhythmic manner, two or more waves occurring during the 24-hour period.

2. Nuclear and cell division proceed in a similar rhythmic fashion.

3. The times of occurrence of maxima and minima are dependent upon the time of initiation of metabolic activity and not upon the time of day by the clock.

4. Elongation and cell division, as regards time of maxima and minima, are, in general, reciprocals of each other.

5. This reciprocal relation existing between elongation and cell division accounts for a large share, at least, of the rhythms found in these plants.

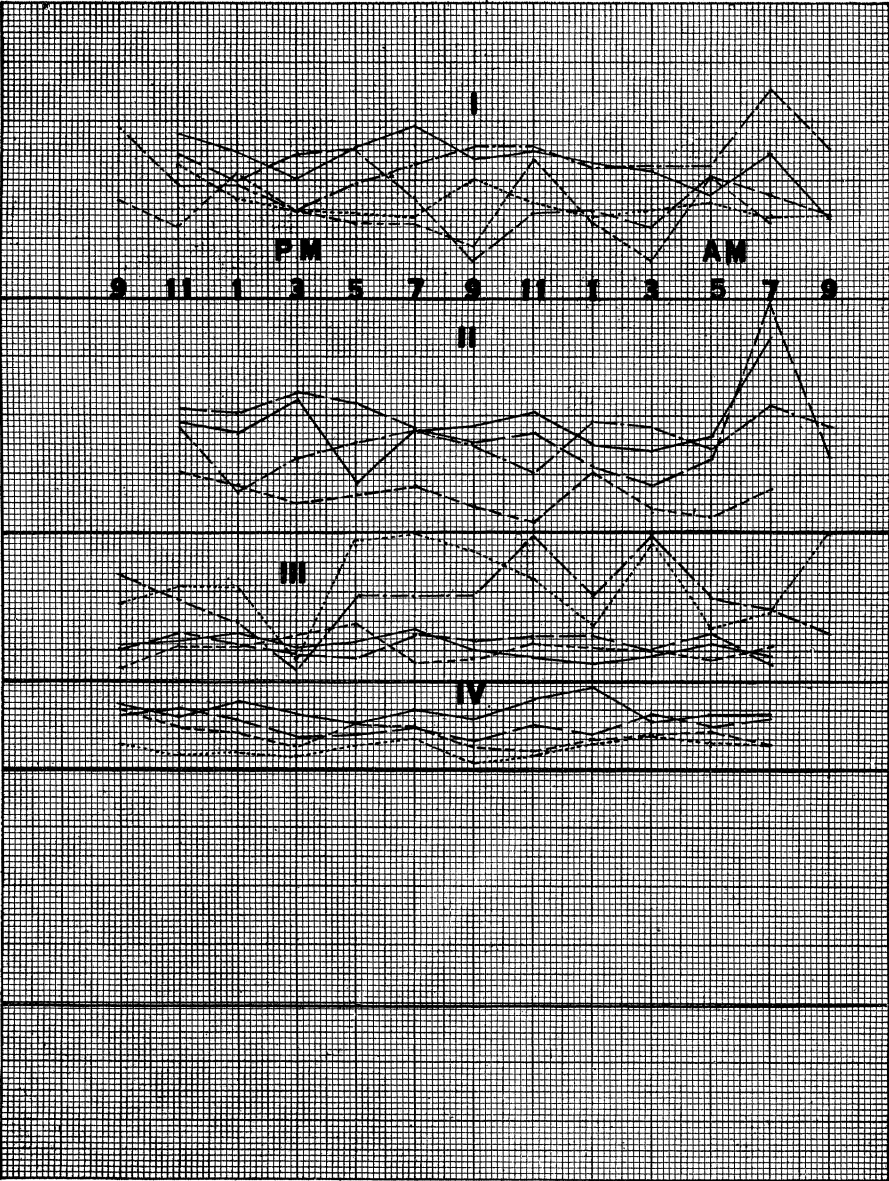
The writer desires to take this opportunity of expressing his appreciation to Professor F. C. Newcombe, under whose direction this work was done, for his constant encouragement and helpful criticism; also to Professor J. B. Pollock and Professor R. M. Holman for helpful criticism and suggestions.

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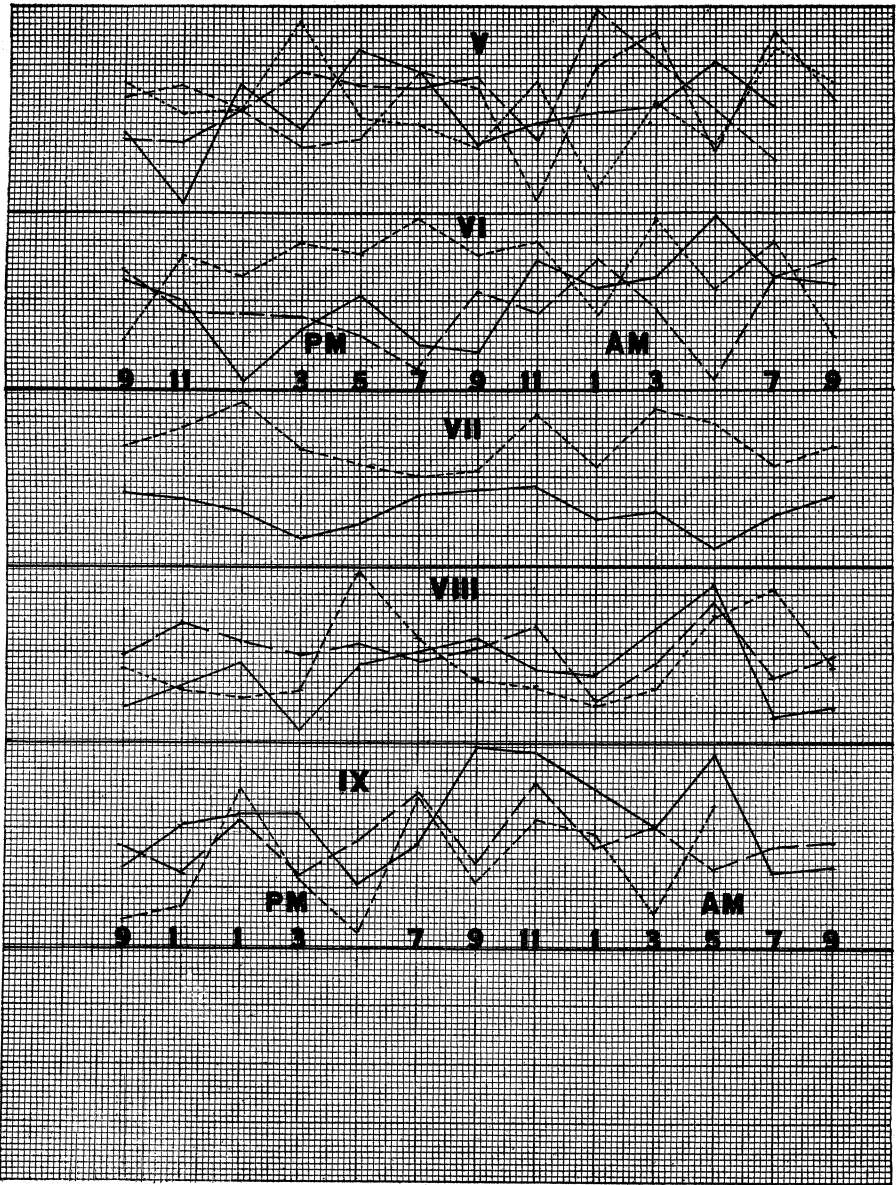
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FRIESNER: DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN ROOTS.



FRIESNER: DAILY RHYTHMS OF ELONGATION AND CELL DIVISION IN ROOTS.

EXPLANATION OF PLATES XXIV AND XXV

Ordinates in curves I-IV show rate of elongation in mm. per hour; in curves V-IX they show the number of cells per sq. mm. undergoing mitosis. The abscissae show time of day by the clock.

I

(Base line = 0. Scale, 1 square = .08 mm.)

193. (————)	Elongation of <i>Pisum sativum</i> .	Germination at 9 A.M.
194. (-----)	“ “ “ “	“ “ “
160. (-----)	“ “ “ “	“ “ 6 P.M.
174. (.....)	“ “ “ “	“ “ 8 P.M.
102. (— . —)	“ “ <i>Zea everta</i> .	

II

(Base line = 0. Scale, 1 square = .08 mm.)

70. (— — —)	Elongation of <i>Lupinus albus</i> .	Germination at 9 A.M.
73. (————)	“ “ “ “	“ “ “
272. (-----)	“ “ <i>Allium Cepa</i> (bulb)	“ “ “
296. (— . —)	“ “ “ “	“ “ “

III

(Base line = 0. Scale, 1 square = 0.1 mm.)

275. (————)	Elongation of <i>Allium Cepa</i> (seed).	Germination at 9 A.M.
276. (-----)	“ “ “ “	“ “ “
288. (-----)	“ “ “ “	“ “ “
III. (— . —)	“ “ <i>Cucurbita Pepo</i>	“ “ “
III. (.....)	“ “ “ “	“ “ “

IV

(Base line = 0. Scale, 1 square = .08 mm.)

273. (————)	Elongation of <i>Allium Cepa</i> (seed).	Germination at 9 A.M.
274. (-----)	“ “ “ “	“ “ “
281. (-----)	“ “ “ “	“ “ “
283. (.....)	“ “ “ “	“ “ “

V

(Base line = 125. Scale, 1 square = 10 cells.)

2. (————)	Mitosis in <i>Pisum sativum</i> (wrinkled)	Germination 9 A.M.
27. (-----)	“ “ “ “ (smooth)	“ “ “
28. (-----)	“ “ “ “	“ “ 2 P.M.
31. (.....)	“ “ “ “	“ “ 8 “

VI

(Base line = 125. Scale, 1 square = 15 cells)

33. (————)	Mitosis in <i>Pisum</i> (smooth).	From refrigerator at 9 A.M.
35. (-----)	“ “ “ “	“ “ 1 P.M.
7. (-----)	“ “ <i>Zea everta</i> .	Germination at 9 A.M.

VII

(Base line = 0. Scale, 1 square = 5 cells.)

1. (————)	Mitosis in <i>Lupinus albus</i> .	Germination at 9 A.M.
13. (-----)	“ “ “ “	“ “ “

VIII

(Base line = 50. Scale, 1 square = 10 cells.)

10. (————)	Mitosis in <i>Allium Cepa</i> (bulb).	Germination at 9 A.M.
24. (-----)	“ “ “ “	“ “ “
5. (-----)	“ “ <i>Vicia faba</i> .	Germination at 9 A.M.

IX

(Base line = 300. Scale, 1 square = 10.)

12. (————)	Mitosis in <i>Allium Cepa</i> (seed).	Germination 9 A.M.
22. (-----)	“ “ <i>Allium canadense</i>	“ “ “
23. (-----)	“ “ <i>Allium cernuum</i>	“ “ “